172

MEMORANDUM OF AGREEMENT

This MEMORANDUM OF AGREEMENT is made on this ---------- day of Two thousand and _____ BY AND BETWEEN President of India, acting through ----------, Department of Biotechnology, Ministry of Science and Technology, Government of India, New Delhi, hereinafter referred to as the 'DBT' (which expression unless excluded by or repugnant to the subject shall mean and include its successor-in-office and assigns) of the ONE PART;

AND

University of Agricultural Sciences, G.K.V.K, Bangalore-65, a state Agricultural University under a ICAR system, having its registered office in/at G.K.V.K, Bangalore-65, Karnataka hereinafter referred to as UASB (which expression shall where the context so admits include its successors and permitted assigns) of the OTHER PART;

WHEREAS DBT being desirous of funding a research project under Bio-CARe Woman Scientist scheme decided to support a project submitted by Dr K.V. Padmalatha for the attainment of the objectives, hereinafter described in the Annexure I annexed hereto;

This Memorandum of Agreement (MoA) defines the role and responsibilities of the participating agencies, monitoring and other matters related to the Comparative Analysis of miRNA expression profiles during fibre development stages in allotetraploid and diploid cotton species (No. BT/Bio-CARe/02/835/2011-12 dated: 04.10.2013).

NOW THE PARTIES HERETO AGREE AS FOLLOWS:-

1.0 . ROLE OF DEPARTMENT OF BIOTECHNOLOGY, NEW DELHI

To provide funds to the extent of Rs. 32,00,000/- over a period of three years from the date of sanction of the project, to the end of the project for undertaking activities as detailed in Annexure 1. Details of the funds to be provided are given in Annexure II.

2.0. ROLE OF UASB (Institute/NGO)

K.V. Padmalatha
(K.V. Padmalatha, PI)

Dr. M. UDAYAKUMAR
Project Co-ordinator
Dep't. of Crop Physiology
UAS, GKVK, Bangalore - 65

19/5/14
PROFESSOR & HEAD
DEPT. OF CROP PHYSIOLOGY
UNIV. AGRI. SCIENCES
G.K.V.K., BANGALORE - 560 065

2.1. To provide their contribution of _____(amount)____ for ------- years from date of sanction of the project as detailed in Annexure – II. *(if a jointly supported project)*: **-NA-**

2.2. To provide existing facilities as mentioned in the project document.

2.3. To be responsible for accomplishing objectives identified and activities listed.

To allow the Scientists authorized by DBT to work with the Research & Development team of the center in all stages of process development and production.

2.4. To recruit all scientific and non-scientific staff as sanctioned by DBT.

2.5. To prepare and submit all periodical reports and other documents that would be required by DBT.

2.6. To maintain a separate audit head of account for the grants received from DBT for the project.

2.7. To submit an annual audited statement of expenditure incurred under the project.

2.8. To ensure effective utilization of the grant given by DBT for the purpose for which it was granted and to ensure timely progress of project work.

2.9. The manpower, both scientific and non-scientific, recruited shall be purely on contractual terms & conditions such that the contract for engagement of the manpower shall run concurrently with the said project period only.

3.0 DURATION OF PROJECT

3.1 Duration of project shall be three years from the date the Project has been sanctioned by DBT.

4.0 RIGHTS OF OWNERSHIP/TECHNOLOGY TRANSFER AND UTILIZATION

4.1 The know-how generated from the project by Dr K.V. Padmalatha (Principal Investigator) will be the joint property of UASB and DBT, Government of India. It shall be the responsibility of Principal Investigator to take necessary action for protection of the intellectual property arising out of the PROJECT through proper instruments, such as, patents, copy rights, etc.

4.2 The know-how developed may be transferred to other entrepreneurs on a non-exclusive basis on such terms and conditions as may be determined by DBT.

4.3 All the assets including the equipment and produce acquired will be the property of DBT and shall not be utilized for purposes other than those for which the grant has been

sanctioned. The rights of ownership under this MoA shall not be transferred to any other party without prior approval in writing of DBT.

4.4 It shall be the responsibility of Principal Investigator to ensure that support of DBT is suitably acknowledged in the publications (papers, reports, etc.) arising out of the PROJECT.

5. SECRECY

It is hereby agreed that the participating agencies shall keep information and data collected completely secret provided that the right to transfer the technology shall rest with the DBT

6. MONITORING

6.1 The progress of implementation of the project and proper utilization of grant shall be reviewed by the DBT and by the Monitoring Committee set up by DBT.

6.2 The periodic progress of physical achievements and the utilization of funds, statement of expenditure shall be evaluated by the Monitoring Committee.

6.3 The Comptroller and Auditor General of India, at his discretion shall have the right of access to the books and accounts of the project (No. BT/Bio-CARe/02/835/2011-12 dated: 04.10.2013) for the grants received from DBT for this project.

6.4 The DBT may terminate the grant at any stage if it is convinced that the grant has not been properly utilized or appropriate progress has not been made. In the event, DBT terminates the grant, Principal Investigator shall hand over all documents including technical details and equipment purchased related to the project.

7.0 DURATION OF MEMORANDUM OF AGREEMENT

This MoA will remain inforce for the duration of the project and until all claims are settled between DBT and UASB

8.0 ARBITRATION

In the event of any question, dispute or difference whatsoever arising between the parties to this Agreement out of or relating to the construction, meaning, scope, operation or effect of this Agreement or the validity of the breach thereof shall be referred to an Arbitrator to be appointed by mutual consent of both the parties herein. If the parties cannot agree on the appointment of the Arbitrator within a period of one month from the notification by one party to the other of existence of such dispute, then the Arbitrator shall be nominated by the Secretary, Department

of Legal Affairs, Ministry of Law & Justice, Government of India. The provisions of the Arbitration and Conciliation Act, 1996 will be applicable and the award made thereunder shall be final and binding upon the parties hereto, subject to legal remedies available under the law. Such differences shall be deemed to be a submission to arbitration under the Indian Arbitration and Conciliation Act, 1996, or of any modifications or reenactments thereof.

9.0. GOVERNING LAW

This Contract shall be governed by the Law of India for the time being in force

IN WITNESS WHEREOF the parties hereto have signed, sealed and delivered this Agreement on the day, month and year first above written in presence of:

Witnesses: Signed by ----------

1. ----------

(Designation)

2. For and on behalf of The President of India

Witnesses: Signed by ----------

1. ----------

(Designation)

2.

For and on behalf of

UAS, Bangalore-65, Karnataka

TERMS & CONDITIONS OF THE GRANT

(To be signed and enclosed with concern filled proforma)

1. Approval of the Research proposal and the grant released would be for the specific project mentioned in paras I to V of this proposal and grant should be exclusively spent on the project for which it has been sanctioned within the stipulated time. The Institute is not permitted to seek or utilise funds from any other organisation (Government, Semi Government, Autonomous or Private) for this research project. Any unspent part of amount would be surrendered to the Govt. of India through an account payee demand draft drawn in favour of the "Drawing and Disbursing Officer, Department of Biotechnology, New Delhi", and carry forward of funds of the next financial year for utilization for the same project may be considered only with the specific approval of the Department of Biotechnology (DBT).

2. For permanent/semi-permanent assets acquired solely or mainly out of the grant, an audited record in the form of a register in the prescribed proforma (enclosed at **Appendix-'A'**) shall be maintained by the Institute. The term **"assets"** means (I) immovable property and (II) movable property of a capital nature, where the value exceeds Rs. 1000/- The grant will not be utilised for construction of any immovable property, Full facilities by way of accommodation, etc. for the project will be given by the Institute.

3. All the assets acquired from the grant will be the property of Govt. of India and should not without the prior sanction of the Deptt. of Biotechnology, be disposed of, or encumbered or utilised for purpose other than those for which the grant has been sanctioned.

4. At the conclusion of the project, the Govt. of India will be free to sell or otherwise dispose of assets which are the property of the Government. The Institute shall render to Govt. necessary facilities for arranging the sale / disposal of these assets. **The Government may, however, consider the request of host institutions to retain the assets created under a project for carrying out similar work for the promotion of science.**

5. The implementing Institute/PI will furnish progress report of work on the project every six months. The progress of the project will also be reviewed/monitored at least once a year by the concerned Task Force/Project Monitoring Committee, etc. In addition the DBT shall designate Scientists/Specialists to visit the Institute periodically for reviewing the progress of work and for suggesting such measures as to ensure early realisation of the objectives of the project. On completion of the project five copies of a consolidated report of the work done on the subject would be submitted to the Department of Biotechnology.

6. The Institute is required to send to DBT a list of assets referred to at Sl. No. 2 above at the end of each financial year as well as at the time of seeking further installments of the grant.

7. The Institute would furnish to the Deptt. of Biotechnology a Utilization Certificate (Copy enclosed at **Appendix - 'B'**) and an audited statement of expenditure (**Copy enclosed at Appendix - 'C'**) duly signed by the P.I., the Head of the Institute and the Head of the Finance wing, pertaining to the grant at the end of each financial year as well as a consolidated statement of expenditure at the end of the completion of the project.

8. A stamped receipt be sent to the Deptt. of Biotechnology on receipt of the Cheque/ Demand draft towards each release.

9. The Comptroller and Auditor-General of India at his discretion shall have the right of access to the books and accounts of the Institute for the grant received from the Government.

10. The Institute would maintain separate audited accounts for the project. If it is found expedient to keep a part or whole of the grant in a bank account earning interest, the interest thus earned should be reported to the Deptt. of Biotechnology.

11. Sale proceeds, if any, as a result of the development of the project arising directly from funds granted by the Deptt. of Biotechnology shall be reported to the Govt. of India. The Govt. of India may at its discretion allow a portion of such receipt to be retained by the Institute for its utilisation for the project activities.

12. Investigators/Institutes wishing to publish papers based on the research work done under Deptt. of Biotechnology projects should acknowledge the financial support received from the Deptt. of Biotechnology.

13. Investigators/Institutes may utilize various resources such as the Bioinformatics resources, experimental materials, reagents, cell lines, animals, etc. from the National facilities/Institutes/Centres established by this Department as per the terms of transactions followed by them. More information may be obtained about such facility from DBT websites: http://www.dbtindia.org// www.dbtindia.nic.in, www.btisnet.ac.in.

14. Investigators / Institutes shall follow the detailed instructions on technology transfer and Intellectual Property Rights (IPR) as given at Annexure - V. The same has the approval of the Ministry of Finance, Govt. of India vide Deptt. of Expenditure, Plan Finance II – Division Letter No. 33 (5) /PF.II/99 dated 22nd February, 2000. Any deviation from these instructions may be brought to the notice of this Department.

15. Investigators / Institutes may file patents with the help of the Biotechnology Patents Facilitating Cell (BPFC) established at DBT on priority bases. The format for filing the patents may be seen at Annexure –VI.

16. The Govt. of India (Deptt. of Biotechnology) will have the right to call for drawings, specifications and other data necessary to enable the transfer of know-how to other parties and the Institute shall supply all the needed information at the request of the Department of Biotechnology which will ensure confidentiality. The information required for commercializing Biotechnologies may be furnished to this Deptt. as per the format enclosed at Annexure – VII. More information on commercialization can be found at the website www.ebc.nic.in.

17. The Institute may not entrust the implementation of the work for which the grant is being sanctioned to another institution and to divert the grant receipts as assistance to the latter institution. However, in such situations the express permission of DBT may be obtained. In case the grantee is not in a position to execute or complete the project, it may be required to refund forthwith to the Govt. of India (Department of Biotechnology) the entire amount of grant received by it.

18. The human resources that may be engaged for the project by the Institute are not to be treated as employees of the Govt. of India and the deployment of such human resource at the time of completion or termination of project, will not be the concern/responsibility of the Govt. of India. The Organisation may make reservations for Scheduled Castes, Schedule Tribes etc. in the human resource to be engaged for the project in accordance with the instruction issued by the Govt. of India from time to time.

19. The Deptt. of Biotechnology reserves the right to terminate the grant at any stage and also to recover the amounts already paid if it is convinced that the grant has not been properly utilized or the work on the project has been suspended for any unduly long period or appropriate progress is not being made.

20. The project will become operative with effect from the date of release of the first installment for the project.

21. If the Investigator to whom a grant for a project has been sanctioned leaves the institution where the project is being implemented, he shall submit five copies of complete and detailed report of the work done by him on the project and the money spent till the date of his/her release and shall also arrange to refund the unspent balance, if any.

PROFORMA – I

PROFORMA FOR SUBMISSION OF PROJECT PROPOSALS ON RESEARCH AND DEVELOPMENT, PROGRAMME SUPPORT

(To be filled by the applicant)

PART I: GENERAL INFORMATION

1. Name of the Institute/University/Organisation submitting the Project Proposal : University of Agricultural Sciences, Gandhi Krishi Vignyan Kendra, Bangalore-560 065.

2. State : Karnataka

3. Status of the Institute : State University

4. Name and designation of the Executive Authority of the Institute/University forwarding the application : Dr H. Shivanna, Director of Research, University of Agricultural Sciences, GKVK, Bangalore, Karnataka.

5. Project Title : Comparative analysis of miRNA expression profiles during fibre development stages in allotetraploid and diploid cotton species.

6. Category of the Project : R&D

7. Specific Area : Agriculture and Allied areas

8. Duration : Three years

9. Total Cost : Rs. 49,27,750

10. Is the project Single Institutional or Multiple-Institutional (S/M) ? : Single Institutional

11. If the project is multi-institutional, please furnish the following : -NA-

12. Scope of application indicating anticipated product and processes :

From the present study, potential microRNAs (miRNAs) and their target genes involved in fibre development will be identified to improve fibre properties in cultivated cotton species.

13. Project Summary (Not to exceed one page. Please use separate sheet) : Attached (Encloser-1)

PART II: PARTICULARS OF INVESTIGATORS

(*One or more co-investigators are preferred in every project. Inclusion of co-investigator(s) is mandatory for investigators retiring before completion of the project*)

Principal Investigator:

14. **Name:** K.V. PADMALATHA

    Date of Birth: 30.07.1976

    Sex (M/F): Female

    Designation: Research Associate

    Department: Plant Biotechnology

    Institute/University: NRC on Plant Biotechnology

    Address: NRC on Plant Biotechnology, Pusa Campus, IARI, New Delhi

    PIN: 110012

    Telephone: 09953789208

    E-mail: kv.padmalatha@gmail.com

    Number of research projects being handled at present: -NA-

15. **Co-Investigator:** -NA-

16. **Co-Investigator:** -NA-

PART III : TECHNICAL DETAILS OF PROJECT

(Under the following heads on separate sheets)

16. Introduction (not to exeed 2 pages or 1000 words)

16.1 Origin of the proposal

Cotton fibers are trichome-like single cells derived from the epidermis of the outer seed coat and are excellent model systems to study the primary and secondary cell wall biosynthesis. Cotton fibres undergo four distinctive, but overlapping, developmental stages: initiation, elongation, cellulose biosynthesis and maturation. Out of four cultivated cotton species, two are allotetraploids with AADD genome (*Gossypium hirsutum* and *G. barbadense*) and two diploids with AA genome (*G. herbaceum* and *G. arboreum*). *G. raimondii* is closely related DD genome progenitor for the allotetraploids. Among the cultivated species *G. hirsutum* represents over 95% of the annual cotton crop worldwide. The allotetraploids produce more abundant and higher quality fibres than the extant descendant species, suggesting strong selection on polyploid cotton for fibre properties. *G. barbadense* offers superior fiber quality properties like length, fineness, and strength, while *G. hirsutum* is characterized by high yield. Breeding programs around the world are working towards developing high-yielding *G. hirsutum* cultivars with the fiber properties of *G. barbadense*. *G. herbaceum* and *G. arboreum* are more tolerant to biotic and abiotic stresses but having poor fibre qualities. Therefore, these species can be used to identify molecular and genetic basis of fibre qualities which will be very important for improved breeding to meet global demands for cotton.

High through-put transcriptome and proteome studies in both allotetraploid and diploid cotton species identified several key genes involved in fibre development (Alabady et al., 2008; Yang et al., 2008; Al-Ghazi et al., 2009). Although these studies have elucidated many aspects of fibre development and revealed important candidate genes expressed during developmental phases, many aspects of fibre cell differentiation and elongation remain unexplored. Recent studies suggest that microRNAs (miRNAs) may play essential roles in regulating fibre cell development (Barozai et al., 2008; Wang et al., 2011). miRNAs are known to regulate a wide variety of biological processes, such as development, organ differentiation and stress response (Yang et al., 2007; Sun, 2012). The present study is proposed to identify the potential miRNAs involved in fibre development to improve fibre properties in cultivated cotton species.

16.2 (a) Rationale of the study supported by cited literature (b) Hypothesis (c) Key questions.

A better understanding of the molecular and genetic basis for the physical differences in the fibres in tetraploid (*G. hirsutum* and *G. barbadense*) and their closely related progenitor diploid cotton species (*G. arboreum* and *G. raimondii*) will facilitate the breeding of higher quality *G. hirsutum* varieties and perhaps provide transgenic solutions for improving cotton fibre quality. Several transcriptome and proteome studies have been carried out in *G. hirsutum* to identify the molecular mechanisms involved in the fibre development (Gou et al., 2007; Yang et al., 2008). However, very little information has been accumulated on the differences in fiber gene expression between different tetraploid and diploid cotton species. Recently, only two reports have focused on the transcriptional changes between *G. hirsutum* and *G. barbadense* fibers using microarrays (Alabady et al., 2008; Al-Ghazi et al., 2009). However, fiber quality genes are less well-characterized, and little is known about underlying biological causes of these differences in cotton fiber qualities.

The fibre growth is likely under the strict regulation of phytohormonal signals such as auxins, brassinosteroid, ethylene and giberellins as well as important transcription factors such as

MYB and *WRKY* family proteins (Shi et al., 2006; Yang et al., 2006; Walford et al., 2011). Recently, miRNAs have been demonstrated to regulate a variety of transcription factors and phytohormonal regulators that mediate critical morphogenetic events (Barozai et al., 2008; Wang et al., 2011).

So far, very few studies revealed the differential expression of miRNAs during early elongation of the fibre development in *G. hirsutum* by comparing with its isogenic fuzzless-lintless mutant (Kwaket al., 2009; Pang et al., 2009; Wang et al., 2011). However, no systematic study has yet been reported on the differences in expression of miRNAs in economically important cotton species and how those differences might impact on fibre quality. In the present proposed study, a comparative miRNA expression analysis will be carried out in allotetraploid species (*G. hirsutum* and *G. barbadense*) and their closely related diploid progenitors (*G. arboreum* and *G. raimondii*) to identify the potential miRNAs involved in the fibre development and to improve the fibre qualities in cultivated cotton species.

16.5 Current status of research and development in the subject

In cotton few miRNAs and their targets have been identified by computational methods in *G. hirsutum* based on the conserved characterization of miRNAs (Qiu et al., 2007; Zhang et al., 2007; Barozai et al., 2008) and in *G. arboreum* (Wang et al., 2012). As the cotton is not completely sequenced, computational prediction does not make an effective choice for discovering novel cotton miRNAs. Recently developed high-throughput sequencing technologies provide a powerful approach to identify and quantify small RNAs/miRNAs. Using high-throughput sequencing method, the expression profiles of miRNAs were identified in the (*G. hirsutum* and its near isogenic fuzzless-lintless mutant and showed significant differences in expression abundance of miRNAs between the wild-type and mutant (Kwak et al., 2009). Similarly, several miRNAs were identified during fibre initiation stage in *G. hirsutum* and identified potential targets of these miRNAs (Pang et al., 2009; Wang et al., 2011). Although few reports are there on expression pattern of miRNAs in *G. hirsutum* there are no such reports in other cotton species.

16.6 The relevance and expected outcome of the proposed study

a. The expression profiles of miRNAs during fibre initiation and elongation in cultivated tetraploids (*G. hirsutum* and *G. barbadense*) and their closely related diploid progenitors (*G. arboreum* and *G. raimondii*).
b. Differentially expressed miRNAs among the cotton species.
c. miRNA targets involved in fibre development.
d. Potential candidate miRNAs and their targets for further functional validation to improve fibre quality in highly cultivated cotton species.

16.7 Preliminary work done so far:

The expression pattern of few miRNAs was analyzed using qRT-PCR at fibre initiation and elongation stages in *G. hirsutum* cv. MCU5 and its near isogenic fuzzless-lintless mutant (Encloser-2). During this study, we standardized the protocol for extraction of small RNAs from cotton ovules and fibre tissues by doing modifications in mirPremier microRNA Isolation Kit (Sigma, USA). In addition, we also standardized the protocols for reverse transcription (RT) and quantitative real-time PCR (qRT-PCR) techniques by following the protocol given by Wan et al., (2010).

We also studied the miRNA expression profile during fibre development stages in cotton under drought stress. For this, the small RNA libraries were prepared from fibre initiation and elongation stages from drought induced and normal plants and sequencing was done with Illumina HiSeq platform. Data analysis for identification of potential miRNAs and their targets involved in drought stress adaptation is under progress.

Summarizing, my work experience I am in adept in miRNA expression analysis using qRT-PCR and high throughput data analysis. The experience gained will definitely help me in my proposed project. However, no preliminary work has been done regarding the proposed work.

17 Specific objectives

- Analysis of expression profiles of miRNAs during fibre development (initiation and elongation) stages in cultivated allotetraploids (*G. hirsutum* and *G. barbadense*) and their closely related diploid progenitors (*G. arboreum* and *G. raimondii*).

  Total RNA from fibre initiation and elongation stages will be isolated from each species and small RNAs will be separated by 15% denaturing polyacrylamide gel. Adopter ligated small RNA libraries will be sequenced by Illumina HiSeq 1000 platform. The purified small RNA sequences will be analyzed to identify miRNA sequences. The conserved and novel/unique miRNAs will be identified by mapping the small RNA sequences against miRNA database (www.sanger.ac.uk) and cotton gene sequences (ESTs, genome survey sequences (GSS) and draft sequence of *G. Raimondii*), respectively.

- Identification of differentially expressed miRNAs among cotton species and identification of miRNA target genes.

  Based on data analysis statistically significantly differentially expressed miRNAs among the cotton species during fibre development stages will be identified. The miRNA target genes will be predicted using the psRNA Target program (www.noble.org) data analysis.

- Validation of expression of miRNAs by Northern blot and qRT-PCR.

  Stage specific and species specific miRNAs will be further validated by Northern blot and qRT-PCR analyses.

- Experimental validation of miRNA targets.

  RLM-RACE (RNA ligase mediated rapid amplification of the 5'cDNA ends) technique will be followed to confirm the cleavage sites in the predicted miRNA targets.

18. Work Plan:

18.1 Work plan (methodology/experimental design to accomplish the stated aim)

Plant materials

The tetraploid cotton species (*G. hirsutum* and *G. barbadense*) and their progenitor diploid species (*G. arboreum and G. raimondii*) will be grown under green house conditions. Flowers at 1 or 2 days before anthesis will be tied for self-pollination and tagged as a developmental reference point. The stages of pre-anthesis flowers (for example, three days prior to anthesis, -3 dpa/days post anthesis) will be estimated based on flower bud size and shape. Later bolls will be collected at -3 to 12 dpa covering fibre initiation and elongation stages. Harvested bolls will be frozen in liquid nitrogen, and stored at -80°C.

Total RNA and small RNA isolation

Total RNA and small RNAs will be isolated using Spectrum Plant Total RNA Kit and mirPremier microRNA Isolation Kit (Sigma), respectively. For fibre initiation stage complete ovules separated from cotton bolls from -3 to +3 will be pooled and for elongation stage fibre tissue dissected from the cotton ovules of 7-12 dpa will be pooled and used for total RNA isolation. Quality and quantity of the total RNAs will be estimated using Nanodrop spectrophotometer and Bioanalyzer (Agilent Technologies, USA).

Cotton small RNA sequencing

The total RNA prepared from the ovules (initiation stage) and fibres (elongation stage) will be electrphoresed on 15% denaturing polyacrylamide gel (7M urea) with RNA size markers to separate the small RNAs of 17 to 27 nucleotides. Then the purified small RNAs will be ligated to the 3' and 5' RNA adaptors. The purified adopter ligated small RNAs (57-87 nucleotide leanghth) from each sample will be reverse-transcribed to synthesis of cDNA libraries using primer pairs partially corresponding to the RNA adaptors. Then the cDNA libraries will be subjected to high-throughput sequencing using an Illumina HiSeq 1000 platform.

Identification of miRNAs and their target prediction

Raw sequences will be trimmed for low-quality reads and adaptor sequences to get clean small RNA sequences. First, the sequences coding for the other small RNAs such as rRNA, tRNA, snRNA, and snoRNA, will be removed from the small RNA sequences. Then, the unique small RNA sequences will be used to do a BLASTn search against the miRNA database (miRBase/www.sanger.ac.uk) in order to identify conserved miRNAs in different cotton species. Only perfectly matched sequences with one or two mismatches will be considered as conserved miRNAs. After removing the conserved miRNA sequences, the rest of the small RNA sequences will be used to perform BLASTn searches against cotton ESTs (CGI, http://compbio.dfci.harvard.edu), cotton genome survey sequences (GSS) from NCBI and the *G. raimondii* draft genome in order to obtain precursor sequences for novel miRNAs. Potential pre-miRNAs from those corresponding ESTs and genomic sequences will be examined using mireap (http://sourceforge.net/projects/mireap) followed by prediction of secondary structures with MFOLD (http://mfold.rna.albany.edu). Further, the potential target genes of cotton miRNAs will be predicted using the psRNA Target program (www.noble.org).

Validation of miRNAs

Significantly differentially expressed miRNAs and their targets among the cotton species will be validated by Northern blot and quantitative real time PCR (qRT-PCR). Primers for reverse transcription and qRT-PCR will be designed based on Wan et al., (2010) to discriminate the highly homologous miRNAs. In brief, deoxyuridine-incorporated RT oligonucleotide with a secondary structure will be used for enrichment of miRNA specific cDNA and a hemi-nested primers will be used for qRT-PCR.

Experimental miRNA target validation

RLM-RACE (RNA ligase mediated rapid amplification of the 5'cDNA ends) technique will be followed to confirm the cleavage sites in the predicted miRNA targets.

References:

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18.2 Connectivity of the participating institutions and investigators (in case of multi-institutional projects only): -NA-

18.3 Alternate strategies (if the proposed experimental design or method does not work what is the alternate strategy):

Expression profiles of miRNAs will be identified during fibre development stages in different cotton species using Affymetrix GeneChip microRNA Array (http://www.affymetrix.com). This array contains 7815 mature miRNAs from 71 organisms (miRBase v. 11.0), 4 replicate features per miRNA. As substantial number of miRNAs is conserved in different plant species, analysis using these arrays will provide expression pattern of conserved miRNAs during fibre development stages in various cotton species.

19. Timelines: (Please provide quantifiable outputs)

Period of study	Achievable targets
6 months	Cotton plants of tetraploids and their progenitor diploid species will be grown under green house condition and cotton bolls during fibre development (0 to 20 dpa) stages will be collected.
12 months	Total RNA and small RNAs will be isolated. Small RNA libraries of fibre initiation and elongation stages will be constructed and sequenced by following high-throughput method.
18 months	Sequences of small RNAs will be analyzed and stage specifically enriched miRNAs in each species will be identified.
24 months	The miRNA targets will be identified. Validation of expression pattern of miRNAs by Northern and qRT-PCR analysis.
30 months	Continuation of validation of expression pattern of miRNAs with Northern blot and qRT-PCR. *In-vitro* validation of miRNA targets will be carried out.
36 months	Potential miRNAs and their targets will be identified for genetic manipulation to achieve improvement of fibre properties in cultivated cotton species.

20. Name and address of 5 experts in the field

S.No.	Name	Designation	Address
1.	Dr NeetiSanan-Mishra	Staff Research Scientist	Plant Biology: Plant Molecular Biology, International Centre for Genetic Engineering and Biotechnology (ICGEB), Aruna Asaf Ali Marg New Delhi - 110067, India.
2.	Dr Raj Kamal Bhatnagar	Group leader	Plant Biology: Insect Resistance ICGEB Aruna Asaf Ali Marg New Delhi - 110067, India.
3.	Dr Debasis Pattanayak	Principal Scientist	NRC on Plant Biotechnology, Pusa Campus, IARI, New Delhi-110012
4.	Dr Samir V. Sawant	Senior Scientist	CSIR-National Botanical Research Institute (NBRI), Rana Pratap Marg, Lucknow, 226001, UP, India
5.	Dr N. Nataraja Karaba	Associate Professor	Dpt. of Crop Physiology, University of Agricultural Sciences, Gandhi Krishi Vignyan Kendra, Bangalore-560 065.

PART IV: BUDGET PARTICULARS

Budget (In Rupees)

A. Non-Recurring (e.g. equipments, accessories, etc.)

S. No.	Item	Year 1	Year 2	Year 3	Total
-	-	-	-	-	-

Sub-Total (A)

B. Recurring

B.1 Manpower (See guidelines at Annexure-III)

S. No.	Position No.	Consolidated Emolument	Year 1 (April, 2013-March, 2014)	Year 2 (April, 2014-March, 2015)	Year 3 (April, 2015-March, 2016)	Total (April, 2013-March, 2016)
1.	Research Scientist	40,000 per month	4,75,000*	4,80,000	4,80,000	14,35,000

Sub-Total (B.1) = 14,35,000

(*Consolidated emolument with Rs. 35,000 per month (<5 years experience) for the month of April, 2013 and with Rs. 40, 000 per month (>5 years experience) from the month of May, 2013 to March, 2014.)

B.2 Consumables

S. No.	Item	Quantity	Year 1	Year 2	Year 3	Total
1.	Chemicals, plastic wear and glass wear		6,00,000	5,00,000	5,00,000	16,00,000

Sub-Total (B.2) = 16,00,000

Other items	Consolidated Emolument	Year 1	Year 2	Year 3	Total
B.3 Travel		50,000	50,000	50,000	**1,50,000**
B.4 Contingency		9,00,000*	1,00,000	1,00,000	**11,00,000**
B.5 Overhead (If applicable)		3,03,750	1,69,500	1,69,500	**6,42,750**
Sub-total of B (B.1+B.2+B.3+B.4+ B.5)		23,28,750	12,99,500	12,99,500	**49,27,750**
Grand Total (A+B)		**23,28,750**	**12,99,500**	**12,99,500**	**49,27,750**

Justification

B. Recurring
B.1 Manpower: Rs. 14,35,000

Consolidated emolument for three years.

B.2 Consumables: Rs. 16,00,000

For purchasing chemicals, reagents, plastic wear and glass wear for RNA and small RNA isolation, Northern blot and qRT-PCR analysis and validation of miRNA targets etc.

B.3 Travel: Rs. 1,50,000

To attend conferences and workshops and for annual work presentations.

B.4 Contingency: Rs. 11,00,000*

a) *The amount of 8,00,000 is for sequencing of small RNA libraries from tetraploid and diploid cotton samples during fibre development stages using high-throughput sequencing technique. Also for purchasing of bioinformatics related software for sequence data analysis.

b) For purchasing of pots to grow plants and maintenance of cotton plants and for making reports etc.

B.5 Overhead: Rs. 6,42,750

The amount of Rs. 6,42,750 is for institutional charges (15%).

PART V: EXISTING FACILITIES

Resources and additional information

1. Laboratory:
    a. Manpower:
    b. Equipments:
    Centrifuges, Incubators, Nanodrop, PCR machines, Real-time quantitative PCR NGS-sequencer, Radioactive facility, Phosphorimager etc.

2. Other resources such as clinical material, animal house facility, glass house. Experimental garden, pilot plant facility etc.

    Glass houses
    Open fields to grow the plants under field conditions.

PART VI: DECLARATION/CERTIFICATION

It is certified that

a) the research work proposed in the scheme/project does not in any way duplicate the work already done or being carried out elsewhere on the subject.

b) the same project proposal has not been submitted to any other agency for financial support.

c) the emoluments for the manpower proposed are those admissible to persons of corresponding status employed in the institute/university or as per the Ministry of Science & Technology guidelines (Annexure-III)

d) necessary provision for the scheme/project will be made in the Institute/University/State budget in anticipation of the sanction of the scheme/project.

e) if the project involves the utilisation of genetically engineered organisms, we agree to submit an application through our Institutional Biosafety Committee. We also declare that while conducting experiments, the Biosafety Guidelines of the Department of Biotechnology would be followed in toto.

f) if the project involves field trials/experiments/exchange of specimens, etc. we will ensure that ethical clearances would be taken from concerned ethical Committees/Competent authorities and the same would be conveyed to the Department of Biotechnology before implementing the project.

g) it is agreed that any research outcome or intellectual property right(s) on the invention(s) arising out of the project shall be taken in accordance with the instructions issued with the approval of the Ministry of Finance, Department of Expenditure, as contained in Annexure-V.

h) we agree to accept the terms and conditions as enclosed in Annexure-IV. The same is signed and enclosed.

i) the institute/university agrees that the equipment, other basic facilities and such other administrative facilities as per terms and conditions of the grant will be extended to investigator(s) throughout the duration of the project.

j) the Institute assumes to undertake the financial and other management responsibilities of the project.

Signature of Project Coordinator
(applicable only for multi-institutional projects)
Date :

Signature of Executive Authority
of Institute/University with seal
Date :

DIRECTOR OF RESEARCH
University of Agricultural Sciences
GKVK, BANGALORE - 560 065

K. V. Padmalatha
Signature of Principal Investigator :
Date : 22.10.2012

Dr. M. UDAYAKUMAR
Project Co-ordinator
Dept. of Crop Physiology
UAS, GKVK, Bangalore - 65

Signature of Co-Investigator
Date :

Signature of Co-Investigator
Date :

PART VII: PROFORMA FOR BIOGRAPHICAL SKETCH OF INVESTIGATORS

Provide the following information for the key personnel in the order listed on PART II.
Follow this format for each person. **DO NOT EXCEED THREE PAGES**

Name : K.V. Padmalatha
Designation : Research Associate

Department/Institute/University : NRC on Plant Biotechnology (NRCPB), Pusa Campus, New Delhi.

Date of Birth : 30.07.1976, **Sex (M/F) :** Female, **SC/ST :** -NA-
Education (Post-Graduation onwards & Professional Career)

S. No.	Institution Place	Degree Awarded	Year	Field of Study
1.	A.N.G.R. Agricultural University, S.V. Agricultural College, Tirupati, A.P., India	M.Sc. (Ag)	July, 2002	Plant Pathology
2.	Ch. Charan Singh University, Meerut, U.P. India.	Ph.D	3rd May, 2008	Botany/Molecular biology based research (Research work done at Plant Virology Unit, IARI, New Delhi.)

A. Position and Honors
Position and Employment (Starting with the most recent employment)

S. No.	Institution Place	Position	From (Date)	To (date)
1.	NRCPB, Pusa Campus, New Delhi.	Research Associate in the NAIP funded project entitled "Genomics of cotton Boll and Fibre development".	13.03.2008	Till date

Honors/Awards: -NA-

Professional Experience and Training relevant to the Project

To know the role of miRNAs in cotton fibre development, the expression pattern of some of the miRNAs were analyzed using qRT-PCR. Small RNAs were extracted from the *G. hirsutum* cv. MCU5 (WT) and its near isogenic lintless-fuzzless (*fl*) mutant at fibre initiation (0 and 2 dpa), elongation (5 and 10 dpa) and secondary cell wall synthesis (15 and 20 dpa) stages using mirPremier microRNA Isolation Kit (Sigma) (Fig. 1; Encloser-2). The quality and quantity of small RNA were checked with Bioanalyzer (Agilent Technologies, USA). The qRT-PCR analysis was carried out to know the differentially expressed miRNAs in fibre-bearing ovules as compared to *fl*-mutant at various fibre development stages. Primers for cDNA synthesis and qRT-PCR were designed based on Wan et. al., (2010) (Table 1; Encloser-2). We compared the

expression pattern of miRNAs such as miR159, miR156/157, miR160, miR164, miR399, miR2948, miR828 and miR894 between WT and *fl*-mutant at various fibre development stages. For example, we identified down-regulation of miR2948 at fibre initiation and elongation stages in WT as compared to *fl*-mutant (Fig. 2; Encloser-2). The target gene of miR2948 is sucrose synthase (Pang et al., 2009) which is proved to be involved in fibre development (Ruan et al., 2003). Down-regulation of miR2948 in WT indicates the high level expression of sucrose synthase during fibre initiation and elongation stages. Similarly, miR164 was down-regulated during fibre initiation stage and up-regulated at fibre elongation stages in WT (Fig. 2; Encloser-2). The target gene of miR164 is a NAC family transcription factor (Pang et al., 2009). Similarly, we identified some of the stage specifically expressed miRNAs in *G. hirsutum* cv. MCU5 during fibre development. The analysis of expression pattern of miRNA targets is under process.

In another study, to know the role of miRNAs in cotton (*G. hirsutum* cv. Bikanery Nerma) under drought stress, total RNA was extracted from drought induced and normal plants during fibre development stages and small RNA libraries were constructed and deep sequenced using Illumina HiSeq 1000 platform. The analysis of small RNA sequences is under process to identify expression profiles of miRNAs during fibre development stages.

In my previous studies, I analyzed the large scale transcriptome data of cotton (*G. hirsutum* cv. Bikanery Nerma) under drought stress during fibre development stages to identify key genes and pathways involved in drought stress adaptation and to know the adverse effect of drought on fibre development (Padmalatha et al., 2012a). Similarly, a comparative genome-wide transcriptome analysis was carried out during fibre development stages using *G. hirsutum* cv. MCU5 and its fuzzless-lintless mutant to identify fibre preferential genes (Padmalatha et al., 2012b). The knowledge on cotton fibre transcriptome and bioinformatics used for large scale expression data analysis will help me to proper analysis of sequences of small RNAs to identify significantly differentially expressed miRNAs and their target genes.

B. Publications (Numbers only):
Research Papers: Seven
Patents : One
Others (Posters and Abstracts) : Eight

Selected peer-reviewed publications (Ten best publications in chronological order)

1. **Padmalatha K.V**, Patil D.P, Krishan Kumar, Dhandapani G, Kanakachari M, Kumar S, Leelavathi S, Reddy P.S, Neha Jain, Powar K.N, Vamadevaiah H, Katageri I.S, Reddy M.K., Solanke A.U, Vanga Siva Reddy and Polumetla Ananda Kumar (2012b). **Transcriptome analysis of fuzzless-lintless mutant of *Gossypium hirsutum* cv. MCU5 reveals key genes and pathways involved in cotton fibre initiation and elongation.** Under review in BMC Genomics.

2. **Padmalatha K.V**, Dhandapani G, Kanakachari M, Kumar S, Dass A, Patil D.P, Rajamani V, Krishan Kumar, Ranjana P, Bhupendra R, Leelavathi S, Reddy P.S, Neha Jain, Powar K.N, Vamadevaiah H, Katageri I.S, Reddy M.K., Solanke A.U, Vanga Siva Reddy and Polumetla Ananda Kumar (2012a) **Genome-wide transcriptomic analysis of cotton under drought stress reveals significant down-regulation of genes and pathways involved in fibre elongation and up-regulation of stress responsive genes**. Plant Molecular Biology, 78: 223-246.

3. Sadhu Leelavathi, Amit Bhardwaj, Saravanan Kumar, Abhishek Dass, Ranjana Pathak, Shiv S. Pandey, Baishnab C. Tripathy, **K. V. Padmalatha,** Gurusamy Dhandapani, Mogilicherla Kanakachari, Amolkumar U. Solanke, Polumetla Ananda Kumar, Rino Cella, V. Siva Reddy (**2011**) **Genome-wide transcriptome and proteome analyses of tobacco psaA and psbA deletion mutants**. Plant Molecular Biology 76: 407-423.

4. Neha Tiwari, **Padmalatha K.V**, singh V.B, Haq Q.M.I, Malathi (**2010**) **Tomato leaf curl (ToLCBV): infectivity and enhanced pathogenicity with diverse betasatellites**. Archives Virology 155: 1343-1347.

5. Sivalingam, P.N, **Padmalatha, K.V**, Mandal, B, Monga. D, Ajmera, B.D and Malathi, V.G (**2007**) **Detection of begomoviruses by PCR in weeds and crop plants in and around cotton fields infected with cotton leaf curl disease**. Indian Phytopathology 60(3): 356-361.

6. Chatterjee A, Roy A, **Padmalatha K.V**, Malathi V.G, Ghosh S. K (**2005**) **Occurrence of a *Begomovirus* with yellow vein mosaic disease of mesta (*Hibiscus cannabinus* and *Hibiscus sabdariffa*)**. Australasian Plant Pathology 34(4): 609-610.

7. Usharani K.S, Archana Srivastava, **Padmalatha K.V**, Malathi V.G (**2004**) **First report of association of a satellite DNA β molecule with a bipartite begomovirus causing potato leaf curl disease in India**. Journal of Plant Pathology 86 (2): 177-180.

8. Malathi V.G, Usharani K.S, Sivalingam P.N, Rouhibaksh A, **Padmalatha K.V**, Periasamy M (**2004**) **Diversity and complexity of begomoviruses**. Annual Review of Plant Pathology 3: 225-270.

List maximum of five recent publications relevant to the proposed area of work.

1. **Padmalatha K.V**, Patil D.P, Krishan Kumar, Dhandapani G, Kanakachari M, Kumar S, Leelavathi S, Reddy P.S, Neha Jain, Powar K.N, Vamadevaiah H, Katageri I.S, Reddy M.K., Solanke A.U, Vanga Siva Reddy and Polumetla Ananda Kumar (2012). **Transcriptome analysis of fuzzless-lintless mutant of *Gossypium hirsutum* cv. MCU5 reveals key genes and pathways involved in cotton fibre initiation and elongation.** Under review in BMC Genomics.
2. **Padmalatha K.V**, Dhandapani G, Kanakachari M, Kumar S, Dass A, Patil D.P, Rajamani V, Krishan Kumar, Ranjana P, Bhupendra R, Leelavathi S, Reddy P.S, Neha Jain, Powar K.N, Vamadevaiah H, Katageri I.S, Reddy M.K., Solanke A.U, Vanga Siva Reddy and Polumetla Ananda Kumar (2012) **Genome-wide transcriptomic analysis of cotton under drought stress reveals significant down-regulation of genes and pathways involved in fibre elongation and up-regulation of stress responsive genes**. Plant Molecular Biology, 78: 223-246.

C. Research Support: -NA-

Ongoing Research Projects

Sl No.	Title of Project	Funding Agency	Amount	Date of sanction and Duration

Completed Research Projects (State only major projects of last 3 years)

Sl No.	Title of Project	Funding Agency	Amount	Date of completion

K V Padmalatha

Place : New Delhi
Date : 30.10.2012

Signature of Investigator